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Melatonin pathway: breaking the 'high-at-night' rule in trout retina

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Abstract

Pineal melatonin synthesis increases at night in all vertebrates, due to an increase in the activity of arylalkylamine *N*-acetyltransferase (AANAT). Melatonin is also synthesized in the retina of some vertebrates and it is generally assumed that patterns of pineal and retinal AANAT activity and melatonin production are similar, i.e. they exhibit a high-at-night pattern. However, the situation in fish is atypical because in some cases retinal melatonin increases during the day, not the night. Consistent with this, we now report that light increases the activity and abundance of the AANAT expressed in trout retina, AANAT1, at a time when the activity and abundance of pineal AANAT, AANAT2, decreases. Likewise, exposure to darkness causes retinal AANAT protein and activity to decrease coincident with increases in the pineal gland. Rhythmic changes in retinal AANAT protein and activity are 180° out of phase with those of retinal AANAT1 mRNA; all appear to be driven by environmental lighting, not by a circadian oscillator. The atypical high-during-the-day pattern of retinal AANAT1 activity may reflect an evolutionary adaptation that optimizes an autocrine/paracrine signaling role of melatonin in photoadaptation and phototransduction; alternatively, it might reflect an adaptation that broadens and enhances aromatic amine detoxification in the retina.
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Keywords: arylalkylamine N-acetyltransferase; hydroxyindole-O-methyltransferase; melatonin; circadian clock; pineal; retina; fish

1. Introduction

The melatonin pathway (tryptophan → hydroxytryptophan → serotonin → *N*-acetylserotonin → melatonin) occurs in pinealocytes and retinal photoreceptors, reflecting their likely evolution from a common ancestral photodetector (Falcón, 1999; Klein, 2004). Circulating melatonin is always 'high-at-night'; this rhythm serves a signaling function, facilitating the synchronization of physiological rhythms with seasonal changes in the night/day cycle. The daily changes in circulating melatonin are linked very closely to changes in the activity of arylalkylamine *N*-acetyltransferase (AANAT: serotonin → *N*-acetylserotonin, Klein et al., 1997); these changes in AANAT activity control large (~24-hr) changes in the melatonin pathway.

It is generally assumed that retinal AANAT activity exhibits a 24-hr profile similar to that in the pineal gland, i.e. it is 'high-at-night'; it is also assumed that the increase in AANAT activity at night in both tissues serves a signaling role, albeit of a local nature in the retina (Cahill and Besharse, 1995).

The situation in fish is more complex for several reasons. First, two AANAT genes occur in Teleost fish: AANAT1, preferentially expressed in the retina, has broad arylalkylamine substrate specificity and different kinetics from AANAT2, which is preferentially expressed in the pineal gland and has a narrower specificity (Falcón et al., 1996; Coon et al., 1999; Benyassi et al., 2000; Zilberman-Peled et al., 2004). Second, in some cases retinal melatonin increases during the day (Gern et al., 1978; Falcón and Collin, 1991; Zachmann et al., 1991; Garcia-Allegue et al., 2001) in some others during the night (Cahill, 1996; Iigo et al., 1997a,b; 2003). Third, melatonin production in trout retina has been reported to be circadian by one group (Zaunreiter et al., 1998) but to be a direct reflection of photoperiod by another (Mizusawa et al., 2000), as seems to be the case in the pineal of trout (Gern and Greenhouse, 1988; Thibault et al., 1993).

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The study presented here was aimed at determining what mechanisms underlie this reversed pattern of melatonin secretion in trout retina. For this purpose, we investigated daily changes in retinal AANAT1 mRNA, activity and protein in trout maintained under different lighting conditions. This revealed a robust novel 'reversed' rhythm in trout retinal AANAT1 activity, which explains why retinal melatonin is 'high-during-the-day' in this species.

2. Materials and methods

2.1. Animals

Female rainbow trout (*Oncorhynchus mykiss*) were obtained from a commercial source and housed in laboratory conditions resembling their natural habitat with respect to temperature and illumination. Animals were killed by decapitation. The retinas (including neural retina and retinal pigment epithelium) and pineal glands were dissected and frozen in dry ice. Light samples were taken under laboratory fluorescent light, whereas dark samples were dissected under dim red light.

The lighting conditions and sampling times are indicated in the results section and legend of the figures. In the experiments studying the effect of the proteasome pathway inhibitor (MG132), fish (70–100 g b.w.) were anaesthetised in 2-phenoxyethanol (10 ml/40 l water). At 13:00, 20 µl of a solution containing 10^{-4} M MG132 were injected laterally into the eye using an Hamilton syringe. Control animals received an equivalent amount of vehicle (10% dimethylsulfoxide in water). Fish were placed either in the dark or under light for the next 3–4 hr, and then killed.

2.2. AANAT assays

AANAT1 and AANAT2 activities were measured in Spring using validated assays (Benyassi et al., 2000).

2.3. AANAT1 mRNA abundance

Total RNA was extracted using Trizol® as described by the manufacturer (Invitrogen). Total RNA was then fractionated on 1% agarose/0.66 M formaldehyde gel, and subsequently transferred to nitrocellulose membrane (Schleicher and Schuell) by capillary blotting with 10× SSC (3 M NaCl and 0.3 M sodium citrate). RNA was cross-linked to the membrane by heating at 80°C for 2 hr.

A specific AANAT1 probe was prepared by PCR and directed toward the 3′UTR of the trout retinal AANAT1 using bp 930–1465 of GenBank accession number AB007294. The probe was labelled by random priming with [γ-^{32}P]dCTP (3000 Ci mmol^{-1}) (Feinberg and Vogelstein, 1983), and the blots were hybridized as described previously using 20 µg total RNA obtained from a pool of two retinas (Bégay et al., 1998; Coon et al., 1999). Band intensities were measured by integrating the area under the peaks of interest (PhosphoImager/Image Quant; Molecular Dynamics, Sunnyvale, CA). Membranes were also exposed to Hyperfilm (Kodak) at −80°C using intensifying screens. After stripping, the blots were hybridized with a 207 bp human β-actin probe (exons 4 and 5) obtained by PCR amplification of lymphocyte cDNA (Delfau et al., 1990). Each experiment was run in duplicate. Preliminary experiments indicated that this probe detected a single transcript at approximately 1.6 kb in retinal extracts; positive signals were not detected in pineal extracts (not shown), in confirmation of previous findings in the pike (Coon et al., 1999).

2.4. AANAT1 protein

Protein was detected on western blots using rabbit antiserum 3352 raised against synthetic rat $AANAT_{22–37}$ (accession number U38306) phosphorylated at T31 using the catalytic subunit of cyclic AMP-dependent protein kinase and conjugated to cationized bovine serum albumin (BSA) (Ganguly et al., 2005). The antiserum was immunopurified by exposure to sections of blots containing the synthetic peptide; the adsorbed antibody was eluted with 0.1 N acetic acid, pH 2.85, containing BSA (Smith and Fisher, 1984).

Retinal proteins were resolved on Novex pre-formed 14% Tris/glycine gels and transferred to Immobilon-P membrane (Falcón et al., 2001). Immunodetection was done with immunopurified 3352 antiserum (1/250; Falcón et al., 2001), which does not detect unphosphorylated AANAT (Ganguly et al., 2005). Under these conditions, the immunopurified antiserum identifies a major band of trout retinal and pineal protein at ∼23/24 kDa (not shown). A similar band was not identified in extracts from brain, gut, liver, ovaries and testis, whereas high molecular weight bands were also detected in testis extracts (97 kDa and over) (not shown). The AANAT signal in the retina is smaller than that predicted (∼27.7 kDa) from sequence analysis of tAANAT1 (accession number AB007294). This difference may reflect enzymatic cleavage at multiple predicted sites located in the C-terminal tail distal to the PKA phosphorylation/14-3-3 binding site at Ser201; this includes a proline endopeptidase site at Pro208 (http://molbio.info.nih.gov/molbio/molbio_docs/gcg/peptidemap.html). The C-terminal tail may not be protected against proteolytic attack because it would extend from the AANAT/14-3-3 complex (Klein et al., 2002).

2.5. In situ hybridization

Trout retinas were immersion fixed 4 hr in 4% paraformaldehyde in phosphate buffer saline (PBS) at 4°C. After fixation, they were washed in PBS buffer containing, successively, 4% sucrose (5 min), 5% glycerol/10% sucrose (30 min), 10% glycerol/15% (1 hr); they were then placed overnight in 10% glycerol/20% sucrose in PBS. The samples were then embedded in Tissue Freezing Medium (Jung) and frozen (−48°C).

In situ hybridization using digoxigenin-labelled riboprobes was done on 10 µm cryo-sections. Probes were made using a commercially available kit (Roche, France). The AANAT1 probe was generated using the 880 bp cDNA fragment,

corresponding to the coding region of the trout AANAT1 gene (AB007294). A fragment of trout retinal HIOMT was cloned by PCR amplification using degenerate primers (forward: GATTRRCTTCCAKGAAGGRG; reverse: GTCTGCACMARCATGTTCAG) in order to obtain an HIOMT probe. The 186 bp fragment amplified displayed >70% identity/80% similarity with the corresponding sequences from bovine, chicken, human, quail and monkey HIOMT. The hybridization process was as detailed (Thisse et al., 1993) using a probe concentration of 1 μg ml^{-1}, and digoxigenin was immunodetected using a commercially available kit (Roche, France).

In situ hybridization using ^{35}S-labelled oligoprobes (Møller et al., 1997) was done on 16 μm cryo-sections. 38-mer cDNA probes were labelled with ^{35}S-ATP (SA = 1.10^{18} dpm mol^{-1}) using terminal transferase. A mixture of three probes was used for AANAT1: TGATGGAACA–GCGGCCCTGC–ACCTTGAAGC–CAGACTTC, ACTGAGTAAAAAAAG–ATTTA–CACATCAGCA–GGCTCAGG, and TGATGGAACAGCGGCCCTGCACCTTG–AAGC–CAGACTTC. A mixture of two probes was used for HIOMT: GAATATAGAG–GTCAGCATCT–GGCAGTGCAT–CTTTGAAA and GACCCGTCTT–CACACAGCAA–CGCCTCTACC–ACCAACAC. The sections were rehydrated and treated with proteinase K. After delipidation, sections were hybridized overnight at 42°C in a hybridization buffer with the labelled probes (10^7 dpm ml^{-1} hybridization buffer). The sections were then washed in SSC and distilled water and either exposed to an X-ray film for 2–6 weeks or dipped into Amersham LM-1^R emulsion and exposed for 4–8 weeks at 4°C. The number of grains above the photoreceptor layer was counted in an Axiophot microscope with a grid in the ocular.

2.6. Statistics

Each assay was run in duplicate, and each experiment was repeated at least once. Data were submitted to analysis of variance (Anova) followed by Tukey's or Bonferroni's post-test for paired comparison of means, where appropriate. Drawings and statistics were performed using the Prism 4 (GraphPad™) software.

3. Results

Trout retinal AANAT1 activity is low throughout the night and increases during the light period, i.e. it exhibits a 'reversed' pattern relative to that in the pineal gland (Fig. 1). Retinal AANAT1 activity was elevated throughout 24-hr in constant lighting (LL) and suppressed in constant darkness (DD) (Fig. 1).

Photic regulation of trout retinal AANAT1 was examined using atypical light/dark transitions in which animals were exposed at midday to a dark pulse (Fig. 2). Retinal AANAT1 activity decreased following dark exposure and then increased following re-exposure to light. In striking contrast, pineal AANAT2 activity changed in a directly opposite manner as expected from data obtained in previous studies (Thibault et al., 1993).

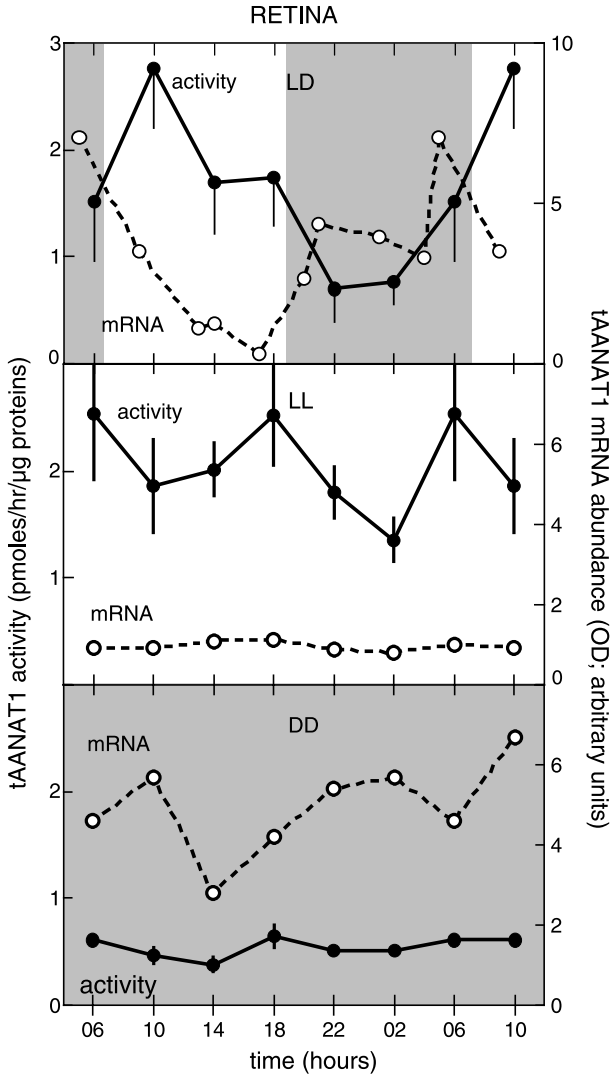

Fig. 1. tAANAT1 mRNA and activity under 24 hr LD, LL or DD cycles. After a period of 5 days under a 24 hr $12L_{(0630–1830)}$/12D cycle, trout were maintained under LD, LL or DD lighting for 24 hr, and killed at the indicated times. The values of AANAT1 mRNA represent the means of duplicate determinations for one experiment; results were confirmed in another experiment. The values of AANAT1 activity are the mean ± SEM ($n = 5$). There were significant variations in LD ($F_{LD} = 2.83$, $P < 0.046$), not in LL ($F_{LL} = 1.8$, $P < 0.16$) nor DD ($F_{DD} = 1.47$, $P < 0.22$) according to ANOVA analysis. Results were confirmed in two other independent experiments.

Changes in AANAT1 activity did not parallel changes in AANAT1 mRNA (Fig. 1); rather, an inverse relationship was found to exist in LD, LL and DD (Fig. 1). A rhythm was not apparent with retinas from trout maintained under either DD or LL (Fig. 1), indicating that environmental lighting generated the AANAT1 mRNA rhythm. Consistent with this, we observed that AANAT1 mRNA was higher under DD relative to LL.

There is a good correlation between the levels of AANAT activity and abundance of AANAT protein in the pineal gland and retina of several vertebrates. Consistent with this, we found that the amount of immunodetected retinal AANAT1 was higher when the activity was high than when the activity was

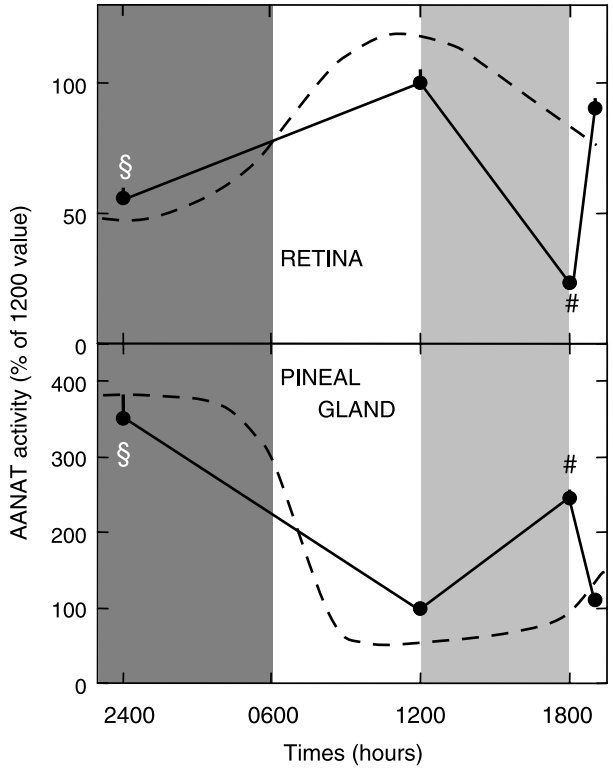

Fig. 2. Effects of atypical light/dark lighting on retinal tAANAT1 and pineal tAANAT2 activities. Trout were adapted to a 12L/12D cycle. Tissues were obtained at midnight (2400) of the scotophase (dark box), at noon (1200) after 6 hr of light, after a subsequent 6 hr dark period during day (gray box; 1800) and after an additional hour of exposure to light (1900). The dashed lines represent daily changes in AANAT activity rhythms based on the data in Fig. 1 (retina) and in previous publications (pineal gland; Thibault et al., 1993). Light suppresses pineal AANAT2 activity, while it induces an increase in the retinal AANAT1 activity. Mean ± SEM ($n=5$); retina: $F=82$, $P<0.0001$; pineal gland: $F=45$, $P<0.0001$; means with different labels are significantly different (ANOVA followed by Newman–Keuls test). Results were confirmed in another independent experiment.

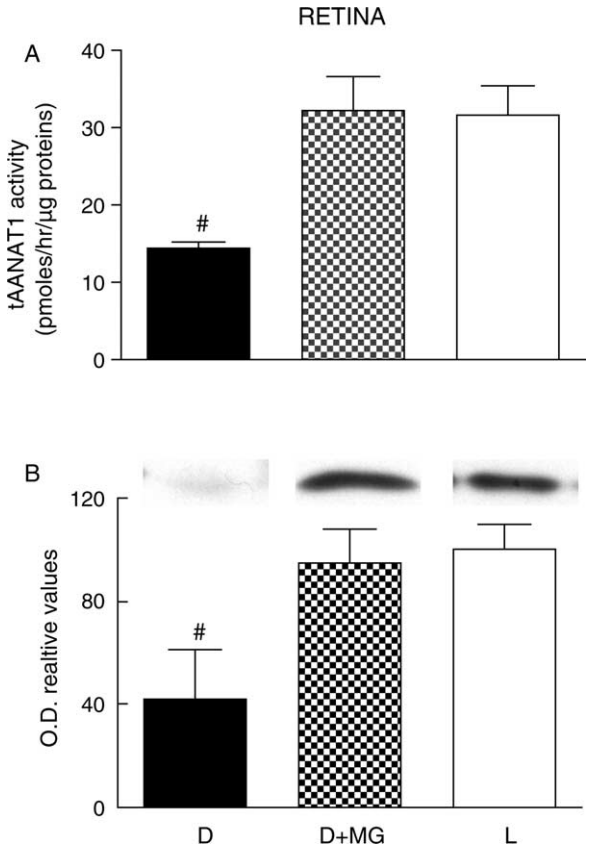

Fig. 3. Effects of a proteasomal inhibitor on trout AANAT1 activity (A) and protein (B). The eyes of trout were injected at 13:00 with 20 µl of either vehicle, or a 10^{-4} M solution of the proteasomal proteolysis inhibitor MG132 (MG). Animals were then placed in the dark (D) or under light (L) for 3 hr. (A) tAANAT1 activity; mean ± SEM ($n=10$) from three independent experiments. Anova indicated that variation of means was statistically significant ($P<0.0001$; # Bonferroni's post-comparison of means: $P<0.01$). (B) After AANAT1 protein was immunodetected, optical densities in the spots were measured, and values were normalized to the value obtained during day. Mean ± SEM ($n=4$) from two independent experiments; Anova-indicated variation of means was statistically significant ($P<0.0001$; # Bonferroni's post-comparison of means: $P<0.05$). There is a positive correlation between AANAT1 activity values and AANAT1 protein; the proteasomal inhibitor prevented the inhibitory effects of darkness on AANAT1 activity and protein.

low (Fig. 3). These dynamic changes appear to be a function of rapid protein proteolysis because intra-ocular administration of an inhibitor of proteasomal proteolysis during the night increased retinal AANAT1 protein and activity to daytime levels (Fig. 3).

The distribution patterns of transcripts encoding AANAT1 and HIOMT, the last enzyme in melatonin synthesis, were studied in trout retina by in situ hybridization. Using non-radioactive probes, both transcripts appeared to be localized in photoreceptor cells as well as in non-photoreceptor cells of the basal inner nuclear layer and ganglion cell layer (Fig. 4). The same cell types expressed both transcripts (compare B with C, and D with E in Fig. 4). Using radioactive probes, AANAT1 and HIOMT mRNAs were detected mainly in photoreceptor cells and AANAT1 expression was three-fold higher at night vs. day, whereas HIOMT expression increased only slightly at night (Fig. 5). The finding that AANAT1 mRNA is higher during the night in sections from trout retina is in agreement with the northern blot analysis of total retinal mRNA (Fig. 1).

4. Discussion

The data in this report provide novel insights into the photoperiodic control of fish retinal AANAT. Most significantly, they contradict the assumption that retinal AANAT is a 'nocturnal' enzyme. Our findings that trout retinal AANAT1 activity is low throughout the night and increases during the light period indicate activity is positively linked to light, not darkness. This is further supported by the observations that AANAT1 activity is (i) elevated throughout 24-hr in constant lighting (LL) and suppressed in constant darkness (DD), and (ii) decreased by unexpected dark during day. Our data explain earlier observations indicating that retinal melatonin content is higher during day than during night in trout (Gern et al., 1978).

It is also evident that a distinctly different relationship between mRNA levels and AANAT1 activity exists in the trout

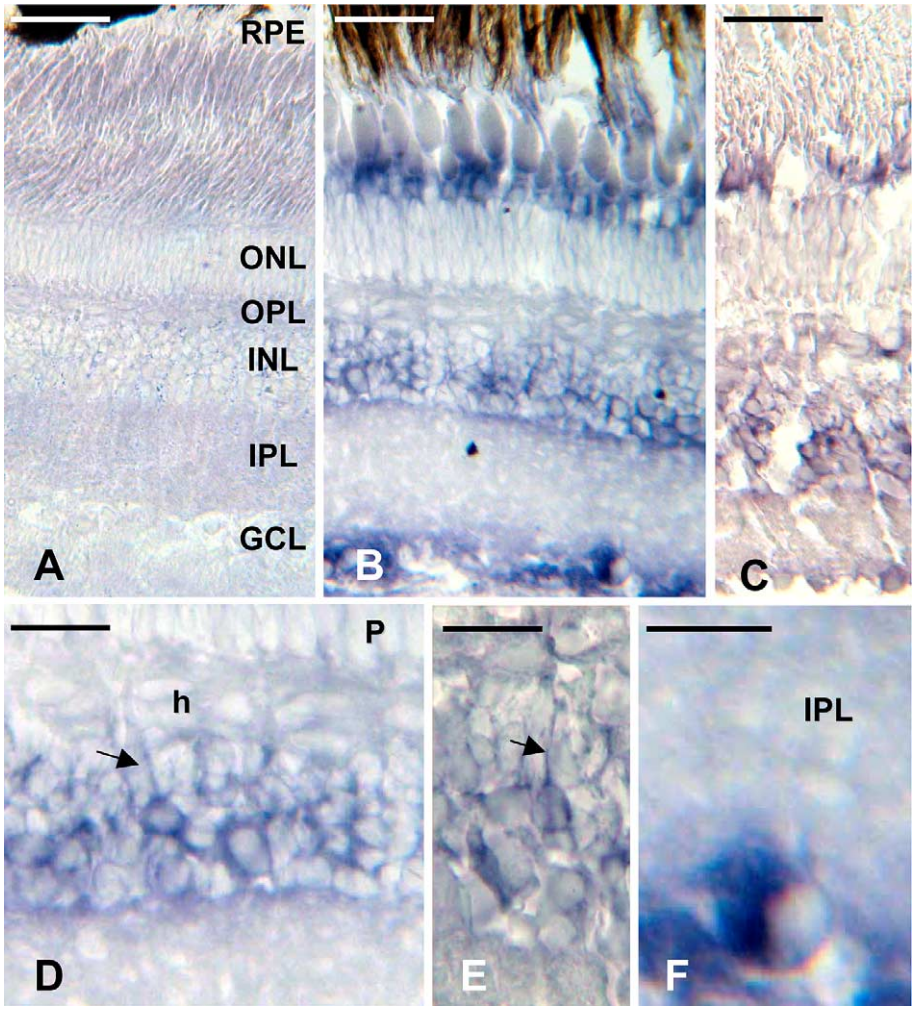

Fig. 4. Localization of AANAT1 and HIOMT transcripts in trout retina by in situ hybridization. (A) AANAT1 sense probe; no labelling is seen. (B–F) Antisense probes. (B) AANAT1. Positive reactions are detected in the apical region of photoreceptor cells; in the outer nuclear layer (ONL), in a population of cells in the inner part of the inner nuclear layer (INL); and, in ganglion cells (GCL). No reaction is detected in the retinal-pigmented epithelial cells (RPE), outer and inner plexiform layers (ONL, INL). (C) HIOMT. Same as in (B). (D) AANAT1 and (E) HIOMT: higher magnification in the inner part of the inner nuclear layer. Horizontal cells (h) in the outer part of the INL, and the basal part of photoreceptor cells (P) are not labelled. The labelled cells project processes upwards (thin arrows) and downwards (thick arrows); some are located above unlabelled amacrine cells; others are close to the inner plexiform layer (thick arrows). (F) AANAT1. High magnification of the ganglion cell labelled in B (right). Bars 50 μm (A–C), 25 μm (D,E), and 20 μm (F).

retina as compared to that in the pineal gland. Measurement of retinal tAANAT1 mRNA revealed that retinal tAANAT1 mRNA abundance was higher in retinas sampled at night, compared to retinas sampled during day, consistent with previous findings (Mizusawa et al., 2000). Our finding of a greater amplitude than previously reported may reflect different strains, photoperiodic conditions, detection method or probes. It is of special interest that the dynamic changes in AANAT1 mRNA in response to environmental lighting seen in the retina are not seen in the AANAT2 mRNA in the pineal gland (Bégay et al., 1998).

This evidence indicates that retinal AANAT1 is under photic regulation, whereas pineal AANAT2 expression is not (Bégay et al., 1998). Moreover, it appears that a circadian clock does not control retinal AANAT1. The absence of evidence of circadian control of trout retinal AANAT1 is generally consistent with the conclusion that a circadian clock does not play a controlling role in melatonin synthesis in trout, based on the studies of the pineal gland (Gern and Greenhouse, 1988; Thibault et al., 1993). Rather, in both tissues, photic control appears to be the primary regulator. This is in sharp contrast to most vertebrate AANAT regulatory systems, which involve a circadian clock (Klein et al., 1997; Falcón, 1999). In these systems, acute photic control, seen as acute photic suppression, can only be demonstrated at night when the circadian clock increases AANAT activity and protein.

The finding that dynamic changes in AANAT activity are not linked to changes in AANAT mRNA in the trout retina is consistent with observations in other species, including the rhesus monkey and sheep, in which AANAT activity and protein exhibit large daily changes in the presence of tonic levels of AANAT mRNA (Klein et al., 1997). This emphasizes that changes in mRNA are not entirely reliable predictors of changes in protein in the case of AANAT and all proteins.

It is notable that an important feature of AANAT regulation is conserved—the role of proteolysis. As alluded above,

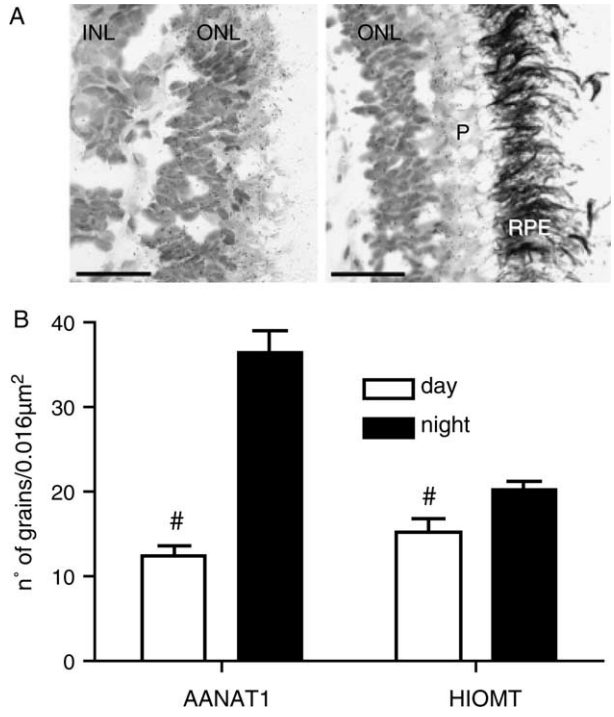

Fig. 5. Quantification of mRNA encoding AANAT1 and HIOMT in the trout retina using in situ hybridization with radioactive-labelled oligoprobes. (A) Photomicrograph of a retina from a trout killed during night-time (left) and daytime (right). AANAT1 hybridization is intense above the outer nuclear layer (ONL) and apical pole of the photoreceptor cells (P). Silver grains were not detected in inner nuclear layer (INL) or retinal-pigmented epithelium (RPE). (B) Number of grains above photoreceptors in trout retina hybridized for AANAT1 and HIOMT mRNA in animals killed during daytime or night-time. The night-time increase is statistically significant for AANAT1 and HIOMT mRNA (mean $\pm$ SEM, $n=5$; $P<0.03$ in each series). Statistical analysis was done using a Mann–Whitney two-tailed t-test). Bars 50 µm.

AANAT activity and protein in chicken and mammalian retinas and in vertebrate pineal glands (including fish) are controlled by a post-translational mechanism involving cyclic AMP-dependent inhibition of proteasomal proteolysis at night (Gastel et al., 1998; Falcón et al., 2001; Iuvone et al., 2002; Klein et al., 2002). Here we found evidence that both AANAT1 protein amount and activity increased during day in trout retina, and that proteasomal proteolysis plays a role in reducing AANAT1 protein amount and activity at night; indeed, intraocular administration of an inhibitor of proteasomal proteolysis during the night increased retinal AANAT1 protein and activity to daytime levels. In mammals, phosphorylated AANAT (P-AANAT) forms a complex with 14-3-3 proteins (Klein et al., 2002). This is believed to increase enzyme activity and protect against proteolysis. Dephosphorylation and disassociation from 14-3-3 lead to enzyme proteolysis. This could be summarized by the following sequence of reactions: AANAT $\Leftrightarrow$ P-AANAT + 14-3-3 $\Leftrightarrow$ P-AANAT/14-3-3 (Klein et al., 2002). From our results, it would appear that the MG132-induced increase in AANAT reflects an increase in P-AANAT. This may indicate that MG132 blocks proteasomal destruction of either free AANAT or P-AANAT, which would shift the equilibrium to the right, increasing accumulation of P-AANAT and of the P-AANAT/14-3-3 complex; it is also possible that MG132 acts via both mechanisms.

Altogether our data indicate that retinal AANAT1 and pineal AANAT2 are under distinctly different regulation. The signal reversal in the relationship between light and AANAT activity in the retina as compared to the pineal gland may occur at a point between light and a controlling second messenger. Two such messengers, cyclic AMP and Ca^{2+}, are known to interact and regulate melatonin secretion in the chicken retina and fish pineal organ (Falcón, 1999; Iuvone et al., 2005). Light could cause an increase in either. It has been observed that there is an increase in cyclic AMP in the fish pineal gland and retina at both dawn and dusk (Kunz et al., 1986; Falcón and Gaildrat, 1997); the increase in cyclic AMP at dawn may be related to the increase in AANAT activity. Although it is possible that Ca^{2+} may play a regulatory role, a mode of action is not obvious because the modes of action of Ca^{2+} are complex, and the best-known photic effect is a dark-induced increase in Ca^{2+} entry. However, it should also be considered that critical AANAT-controlling changes in cyclic AMP and/or Ca^{2+} could occur in discrete sub-cellular compartments; such sub-cellular changes might not be discernable when total cellular cyclic AMP and Ca^{2+} are measured. Finally, it is possible that the effects of light on trout retinal AANAT are mediated by a mechanism that does not involve Ca^{2+} or cyclic AMP, e.g. translation, post-translational modifications, dephosphorylation.

The question of the functional role of AANAT in the retina is raised by these results. The results of distribution studies suggest there may be a role of AANAT and melatonin in at least three cell types. Published results and those presented here indicate that AANAT mRNA expression is abundant in photoreceptor cells and at distinctly lower levels in non-photoreceptor cells of the retina (Cahill and Besharse, 1995; Coon et al., 2002; Garbarino-Pico et al., 2004; Liu et al., 2004; Iuvone et al., 2005). Much less information is available concerning HIOMT. In the chicken, HIOMT mRNA and HIOMT-like immunoreactivity has been localized in the photoreceptor cells (Wiechmann and Craft, 1993; Guerlotté et al., 1996; Wiechmann, 1996) whereas a few immunoreactive cells have also been identified in the inner nuclear layer. Our finding that HIOMT is expressed in the same cell types expressing AANAT1 in trout retina provides the first unequivocal evidence that the serotonin $\rightarrow$ *N*-acetylserotonin $\rightarrow$ melatonin pathway may be active in multiple cell types; it may explain the presence of a melatonin-like compound in the outer and inner nuclear layers of the pike retina (Falcón and Collin, 1991). However, the relative level of presence of this pathway was found to vary, with the photoreceptor cells appearing to be the most likely site of an active melatonin biosynthesis; in addition, the results of in situ hybridization indicate that AANAT1 expression was three-fold higher during the night than during the day revealing that the rhythm in AANAT1 mRNA detected by northern blot reflected changes in photoreceptor AANAT1 mRNA; a small night/day difference was detected in HIOMT mRNA expression indicating that it is also under photic control.

Our data and the known effects of retinal melatonin (Iuvone et al., 2005) suggest that it has a local autocrine/paracrine-signaling role. It is also possible that both AANAT and HIOMT have another function. The original role of the AANAT/HIOMT system in the ancestral photoreceptor cell may have been detoxification; their acquisition by the photoreceptor was an advantage because this reduces depletion of retinaldehyde through Schiff base formation with arylalkylamines (Klein, 2004). Aromatic amine detoxification via *N*-acetylation prevents further non-specific reactions and promotes elimination, as is the case with arylamine *N*-acetyltransferase. Retinaldehyde is known to react in the retina with the amine group of ethanolamine (Sparrow et al., 2000; 2003; Ben-Shabat et al., 2002) and could also react with arylalkylamines (Klein, 2004). Moreover, the increase in retinal AANAT activity during the day among Salmonids documented here might represent an evolutionary innovation that maximally enhances the ability to detect and obtain food by detoxifying arylalkylamines.

The findings in this report are of interest because they document an atypical pattern of AANAT activity in the trout retina. However, they have broader significance because they support the view that the melatonin pathway and AANAT in particular, play different roles in the retina and pineal gland. Whereas in the pineal gland, they are dedicated primarily to melatonin-based photoneuroendocrine signaling, their role in the retina may be broader and more variable on a species-to-species basis. The high levels of AANAT and insignificant levels of HIOMT in the human and bovine retina (Bernard et al., 1995; Klein et al., 1997; Liu et al., 2004) provide indication that a non-signaling role of AANAT may not be limited to fish and may have important clinical implications.

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